

# Tree species as hosts for arbuscular mycorrhizal and dark septate endophyte fungi

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Received: 2011-05-19; Accepted: 2011-08-02

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**Abstract:** A survey of 35 tree species (belonging to 28 genera in 19 families) in Aliyar, South India was carried out to ascertain their arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal status. All the tree species examined had AM association. AM and DSE colonization is reported for the first time in 20 and 14 species respectively. Co-occurrence of AM and DSE was observed in 14 (40%) tree species. The extent of DSE colonization was inversely related to the extent of AM fungal colonization. Six tree species had *Arum*-type, 18 had intermediate-type and 11 had typical *Paris*-type AM morphology. AM fungal spore morphotypes belonging to 11 species in two genera were isolated from the rhizosphere soil. AM fungal spore numbers were not related to the extent of AM colonization and *Glomus* dominated spore diversity. AM association individually and along with DSE were found respectively in the 63% and 44% of the economically important tree species. The occurrence of AM and DSE fungal association in economically important indigenous tree species indicates the possibility of exploiting this association in future conservation programmes of these species.

**Key words:** tree species; AM fungi; *Arum*-, *Paris*-, intermediate- type; dark septate endophyte (DSE)

## Introduction

A wide range of non-pathogenic fungi like arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungi colonize tree roots, which are essential for the health and growth of trees. The functions of these fungi include absorption of immobile nutrients from the soil and their translocation to plant roots, modification

of plant water relations and facilitation of interplant transfer of nutrients (George et al. 1992). In addition to this, AM fungi are also known to improve soil structure and protect plants against pathogens and pollutants (Smith and Read 2008). The AM fungus invades a root and sequesters carbon until changes in root ontogeny preclude continued colonization (Allen et al. 1992). Beneficial effects of AM fungi depend on the host combination and environmental conditions (Kiers et al. 2000; Lovelock and Miller 2002). AM fungi can influence competitive interaction among plant species as well as plant community composition (Bever et al. 2002).

Trees in natural ecosystems are associated with ectomycorrhizal and AM fungi, with the latter being more widespread. Short-lived, fast growing, small-seeded tree species with high photosynthetic activity depend more on AM symbiosis to satisfy their increased nutrient demand, compared to long-lived tree hosts (Lovelock et al. 2003). The importance of root fungal association in trees arises from the fact that trees generally have low rooting densities in the soil.

Arbuscular mycorrhizal associations of trees in various forest types were studied like subtropical evergreen forest and arid zones in India (Sharma et al. 1984; Thapar and Khan 1985; Tarafdar and Rao 1990; Thapar and Vijaya 1990; Raman et al. 1992; Santhaguru et al. 1995; Vijaya et al. 1995), tropical forest in Costa Rica (Janos 1980; Lovelock et al. 2003), rainforest in Australia (Gehring and Connell 2006) and peat swamp forest in Indonesia (Tawaraya et al. 2003). In particular, AM fungal association has been documented in few tree species of Western Ghats (Kandasamy et al. 1988; Mohankumar and Mahadevan 1987, 1988; Muthukumar and Udaiyan 2000; Muthukumar et al. 2001a; Khade and Rodrigues 2003).

Several studies indicate that inoculation of tree seedlings with AM fungi under nursery conditions substantially enhance the growth and quality of the seedlings (Muthukumar et al. 2001b; Muthukumar and Udaiyan 2010). Further, AM fungi reduce the necessity for high levels of phosphorus (P) fertilization during early tree seedling establishment and survival of naturally regenerated seedlings (Habte et al. 2001). The capacity of indigenous tree species in association with AM fungi is very important to

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Responsible editor: Hu Yanbo

forest restoration and conservation purpose (Nandakwang et al. 2008).

Dark-septate endophyte (DSE) fungi have melanized hyphal structures, and forms compactly arranged intracellular structures called microsclerotia within host roots. These fungi associate with plants of many families and habitats ranging from tropics to stressful habitats of arctic environments (Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005). DSE fungi coexist with the variety of mycorrhizal forms (Urcelay 2002) and are presumed to be beneficial to host growth and development (Wu and Guo 2008). The objective of this study was to determine the AM and DSE fungal status of certain indigenous and exotic tree species in Aliyar, Western Ghats, Tamil Nadu.

## Materials and methods

### Study site

Aliyar is located near Pollachi (10.47°N, 76.79°E) at the foothills of Valparai in Anamalai range of Western Ghats, southern India. Aliyar is 280–309 m above sea level and receives most of its mean annual rainfall of 907 mm between July and November. The mean maximum and minimum temperature ranges from 28–35°C, and 19–24°C, respectively. Root and soil samples were collected in 2008 at the end of the rainy season (November).

### Sample collection

Root and soil samples collected from randomly selected five individuals of 35 tree species in 28 genera of 19 families growing naturally were examined in this study. Roots (~2 g) were collected by excavating from the trunk to the feeder roots of each tree species to ensure that roots of intended species are collected (Table 1). Roots were gently washed and fixed in FAA (5 ml formalin – 5 ml acetic acid - 90 ml 70% alcohol) solution and transported to the laboratory for processing. Soil adjacent to roots was collected. Soil samples collected from an individual tree were shade dried, packed individually in polythene bags and stored at 4°C until processing. One-half of the soil samples of all the trees from the site was bulked and used for assessing soil chemistry and the other half of the soil sample of the tree was used for the extraction and enumeration of AM fungal spores.

### Determination of soil characters

Soil pH was determined in 1:1, soil: water (v:v) using a digital pH meter, soon after the soil samples were brought to the laboratory. The total nitrogen (N), and total P was determined according to Jackson (1971) and exchangeable potassium (K) was determined after extraction with ammonium acetate (Jackson 1971). The micronutrients Iron (Fe), Manganese (Mn), Zinc (Zn) and Copper (Cu) were determined according to DTPA method (Lindsay and Norvell 1978).

**Table 1. Arbuscular mycorrhizal (AM), dark septate endophyte (DSE) fungal status and AM morphology of tree species in Aliyar, South India.**

Family/ Plant species	Fungal association <sup>a</sup>	AM Type <sup>b</sup>	Plant status <sup>c</sup>
Anacardiaceae			
<i>Mangifera indica</i> L.	AM	Intermediate	E
Apocynaceae			
<i>Alstonia scholaris</i> (L.) R.Br.	AM	Intermediate	E
<i>Tabernaemontana heyneana</i> Wall.	AM*	Paris	--
<i>Wrightia tinctoria</i> (Roxb.) R.Br.	AM*, DSE*	Paris	E
Bignoniaceae			
<i>Spathodea campanulata</i> Beauv.	AM*	Intermediate	E
Bischofiaceae			
<i>Bischofia javanica</i> Blume.	AM*, DSE*	Arum	E
Bombacaceae			
<i>Bombax ceiba</i> L.	AM*, DSE*	Intermediate	E
Caesalpiniaceae			
<i>Peltophorum pterocarpum</i> (DC.) Baker.	AM*	Paris	E
Combretaceae			
<i>Terminalia bellirica</i> (Gaertn.) Roxb.	AM	Paris	E
Euphorbiaceae			
<i>Drypetes elata</i> (Bedd.) Pax & Hoffm.	AM*	Arum	--
<i>Macaranga peltata</i> (Roxb.) Muell.	AM, DSE <sup>#</sup>	Intermediate	E
Lauraceae			
<i>Litsea monopetala</i> (Roxb.) Pers.	AM*, DSE*	Intermediate	--
<i>Litsea</i> sp.	AM	Intermediate	--
<i>Litsea stocksii</i> (Meisner) Hook.	AM*, DSE*	Intermediate	E
Malvaceae			
<i>Thespesia populnea</i> (L.) Soland.	AM	Intermediate	E
Meliaceae			
<i>Aglaia</i> sp.	AM, DSE*	Arum	--
Mimosaceae			
<i>Acacia auriculiformis</i> A. Cunn.ex Benth.	AM	Paris	--
<i>Acacia dealbata</i> Link.	AM, DSE*	Intermediate	--
Moraceae			
<i>Ficus amplissima</i> J.E.Smith.	AM*	Arum	--
<i>Ficus microcarpa</i> L.	AM*	Paris	E
<i>Ficus racemosa</i> L.	AM, DSE*	Paris	E
<i>Ficus</i> sp.	AM	Paris	--
<i>Streblus asper</i> Lour.	AM*, DSE*	Paris	--
Myrtaceae			
<i>Eucalyptus tereticornis</i> Sm.	AM, DSE*	Intermediate	--
<i>Syzygium caryophyllatum</i> (L.) Alston.	AM*, DSE*	Intermediate	
Oleaceae			
<i>Chionanthus mala-elengi</i> Dennst.	AM*, DSE*	Intermediate	--
Papilionaceae			
<i>Dalbergia latifolia</i> Roxb.	AM	Arum	E
<i>Pongamia pinnata</i> (L.) Pierre.	AM, DSE*	Intermediate	--
<i>Pterocarpus santalinus</i> L.	AM*	Paris	E
Rutaceae			
<i>Clausena dentata</i> (Willd.) M.Roem.	AM*	Arum	--
<i>Glycosmis cymosa</i> Kurz.	AM*	Intermediate	--
<i>Glycosmis pentaphylla</i> (Retz.) DC.	AM*	Intermediate	--
<i>Murraya paniculata</i> (L.) Jack.	AM	Paris	--
Salicaceae			
<i>Salix tetrasperma</i> Roxb.	AM*	Intermediate	
Verbenaceae			
<i>Clerodendrum viscosum</i> Vent.	AM*	Intermediate	E

<sup>a</sup>AM, arbuscular mycorrhiza; DSE, dark septate endophyte ; <sup>b</sup>Arum, Paris, Intermediate; Arum- type, Paris- type, Intermediate- type; <sup>c</sup>E, economically important tree (according to Nair and Henry 1983; Henry et al., 1989. \* First report.

### Preparation of roots for AM and DSE assessment

Fixed roots were washed free of FAA and examined under a dissection microscope ( $\times 20$ ) for AM fungal spores attached to roots. After examination, the roots were cut into 1-cm fragments, cleared in 2.5% KOH at 90°C (Koske and Gemma 1989) for 60 min and acidified with 5 N HCl and stained with trypan blue (0.5% in lacto glycerol) overnight. Roots that remained dark after clearing were bleached in alkaline  $\text{H}_2\text{O}_2$  prior to acidification. The stained roots were examined with a compound microscope (Olympus BX 51, Japan) ( $\times 400$ ) for AM and DSE fungal structures and the percentage of root length colonization was estimated according to magnified intersection method (McGonigle et al. 1990).

The AM-morphology was classified as *Arum*-, *Paris*- or intermediate-type based on the intercellular or intracellular presence of the fungal hyphae and their linear or coiled nature following descriptions of Dickson (2004). Since only whole and squashed roots were examined, we could not distinguish the intermediate sub-types described by Dickson (2004). However, wherever the parallel running hyphae were seen intracellularly the morphology was designated as an intermediate-type. The AM and DSE fungal status of each tree species was checked against relevant literatures (Wang and Qiu 2006; Dickson et al. 2007; Jumpponen and Trappe 1998) for any previous information.

### Enumeration and isolation of AM fungal spores

One hundred grams of soil were dispersed in 1 L water, and the suspension was decanted through a series of 710 to 37  $\mu\text{m}$  sieves. The residues in the sieves were washed into beakers. The sievates were dispersed in water and filtered through girded filter papers. Each filter paper was then spread on a Petri dish and scanned under a dissection microscope (BTI Magno MS24, India) at  $\times 40$  magnifications and all intact spores (non-collapsed spores with cytoplasmic contents and free from parasitic attack) were counted. Sporocarps and spore clusters were considered one unit. Intact AM fungal spores were transferred using a wet needle and mounted in polyvinyl alcohol-lacto glycerol with or without Melzer's reagent on glass slides for identification (Schenck and Perez 1990). Spores were identified based on spore morphology and sub cellular characters and compared to original descriptions in Schüssler's web site ([www.lrzmuemchen.de/~schuessler/amphylo/amphylo\\_species.html](http://www.lrzmuemchen.de/~schuessler/amphylo/amphylo_species.html)). Spore morphology was also compared to the culture database established by INVAM (<http://invam.cag.wvu.edu>).

### Statistical analysis

Analysis of variance (ANOVA) was performed to assess the variation among tree species in AM and DSE fungal variables and the means were separated using Duncan's Multiple Range Test ( $p < 0.05$ ). Regression analysis was used to assess the relationship between AM fungal and DSE fungal variables (SPSS, Windows Version 9). Spore numbers were log transformed and

percentage data on root colonization was arcsine transformed prior to analysis.

## Results

### Soil properties

The sandy loam soil had a pH of 7.9 and an EC of 0.15  $\text{dSm}^{-1}$ . The total N, available P, and exchangeable K were 76  $\text{kg}\cdot\text{ha}^{-1}$ , 7.5  $\text{kg}\cdot\text{ha}^{-1}$ , and 170  $\text{kg}\cdot\text{ha}^{-1}$ , respectively. The soil Fe, Mn, Zn and Cu concentrations were 6.20  $\text{mg}\cdot\text{kg}^{-1}$ , 3.18  $\text{mg}\cdot\text{kg}^{-1}$ , 1.24  $\text{mg}\cdot\text{kg}^{-1}$  and 1.41  $\text{mg}\cdot\text{kg}^{-1}$ , respectively.

### Occurrence of AM and DSE fungal association

All the 35 tree species examined were mycorrhizal (Table 1). Only roots containing arbuscules or arbusculate coils were considered AM. The fungal entry into the root was characterized by the formation of appressorium at the root surface, from which the colonization pegs entered the root to form intra radical colonization (Fig 1a, b).

Fourteen of the 35 plant species examined had DSE fungal association. Septate hyphae and microsclerotia characterized DSE fungal association. DSE fungal association was absent in tree species belonging to certain plant families like Moraceae, Apocynaceae, Anacardiaceae, Euphorbiaceae, Bignoniaceae, Combretaceae, Rutaceae, Papilionaceae, Caesalpiniaceae, Verbenaceae, Lauraceae, Salicaceae, and Malvaceae. Except members of Bischofiaceae, Bombacaceae, Meliaceae, Myrtaceae, and Oleaceae roots containing DSE fungal structures also contained AM fungi.

### AM morphology

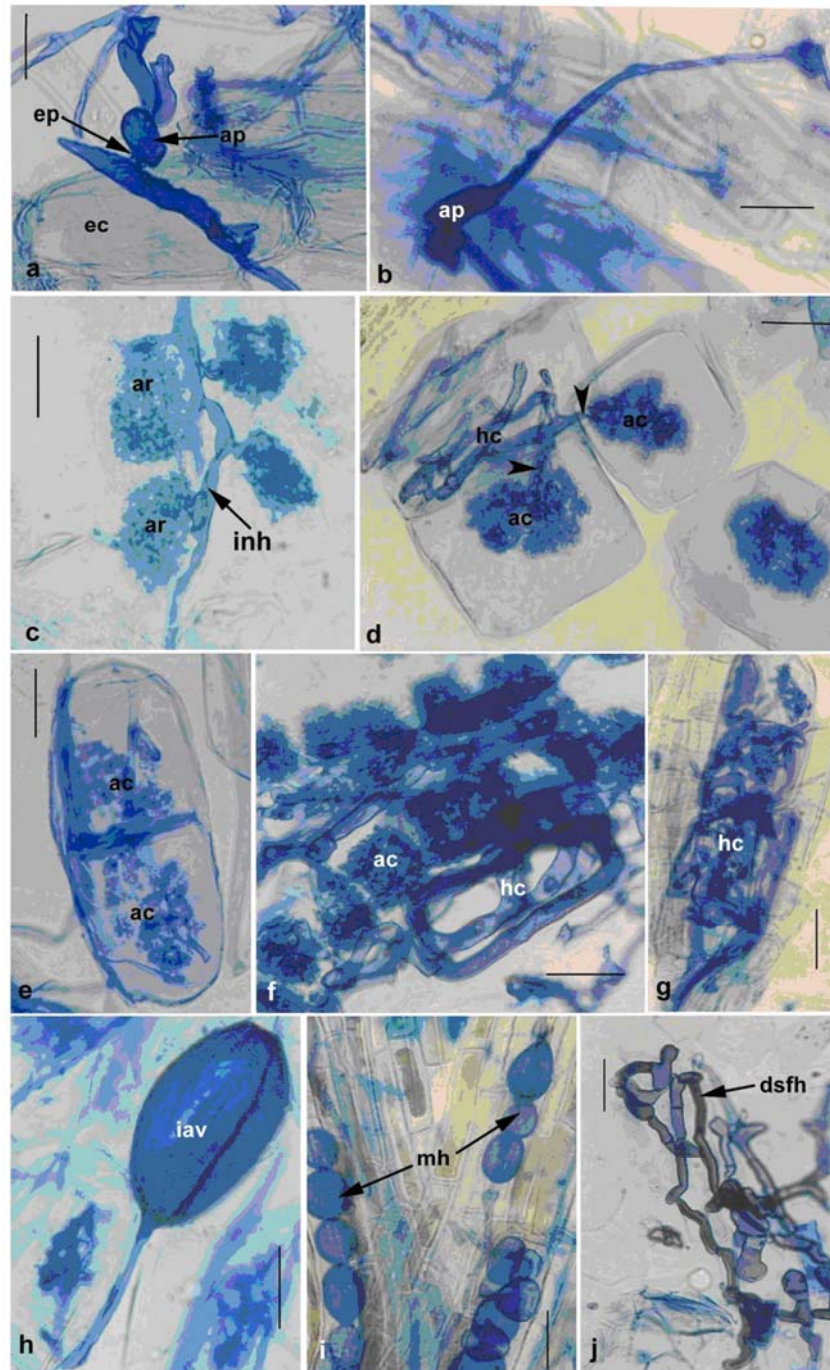
Six tree species had *Arum*-type morphology characterized by the intercellular hyphae, vesicles and intracellular arbuscules (Table 1; Fig 1c, h). *Paris*-type morphology with intracellular hyphal coils, arbusculate coils and intracellular vesicles was found in 11 species (Fig 1g). Eighteen tree species had intermediate-type of AM with intracellular hyphal coils or hyphae along with arbuscules / arbusculate coils and/or inter/intracellular vesicles (Fig 1d-f).

### Extent of AM and DSE association

There were wide differences in the extent of AM fungal colonization and root length with AM fungal structures in different plant species (Table 2). Total root length colonization (%RLTC) ranged between 14 (*Eucalyptus tereticornis*, Myrtaceae) and 70 (*Spathodea campanulata*, Bignoniaceae). The percentage root length with inter- or intracellular hyphae (%RLH) ranged between 3 (*E. tereticornis*, Myrtaceae) and 21 (*Chionanthus malacelengi*, Oleaceae). Similarly percentage root length with arbuscules/ arbusculate coils (%RLA/AC) ranged from 1 (*E. tereticornis* Myrtaceae) to 52 (*Drypetes elata*, Euphorbiaceae) and the

percentage root length with hyphal coils (%RLHC) ranged between 10 (*Litsea stocksii*, Lauraceae) and 65 (*Terminalia bellirica*, Combretaceae). The percentage root length with vesicles (%RLV) ranged from 1 (*Ficus* sp., Moraceae) to 28 (*Macaranga peltata*, Euphorbiaceae). Vesicles were absent in roots of

taxa belonging to Anacardiaceae, Bignoniaceae, Combretaceae, Malvaceae and Salicaceae. There were significant ( $p < 0.01$ ) differences in the extent of root length with mycorrhizal structures among tree species (% RLA,  $F_{34,140}=29.72$ ; % RLV,  $F_{34,140}=3.43$ ; % RLH,  $F_{34,140}=7.36$  and % RLHC,  $F_{34,140}=12.93$ ).



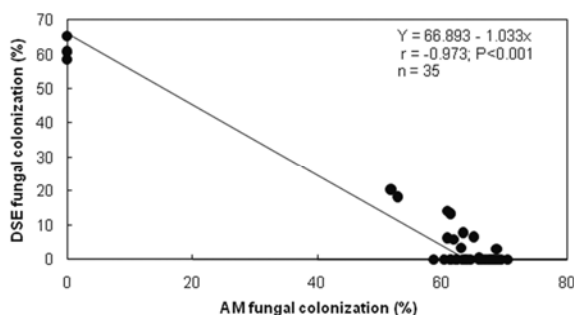
**Fig 1. (a–j) Arbuscular mycorrhizal (AM) morphology and dark septate endophyte (DSE) fungal association in trees of Aliyar.** (a) Entry point (ep) in the epidermal cell (ec) and appressorium (ap) in *Murraya paniculata* (Rutaceae), (b) Appressorium (ap) in *Salix tetrasperma* (Salicaceae), (c) Arbuscules (ar) and intercellular hyphae (inh) in *Drypetes elata* (Euphorbiaceae), (d) Arbusculate coils (ac) and hyphal coil (hc) in *Mangifera indica* (Anacardiaceae), (e) Arbusculate coils (ac) in *Salix tetrasperma* (Salicaceae), (f) Arbusculate (ac) and hyphal coils (hc) in *Thespesia populnea* (Malvaceae), (g) Hyphal coils in *Ficus* sp. (Moraceae), (h) Intra cellular vesicles (iav) in *Dalbergia latifolia* (Papilionaceae), (i) Moniliform hyphae (mh) in *Acacia dealbata* (Mimosaceae) (j) DSE fungal hyphae (dsfh) in *Chionanthus mala-elengi* (Oleaceae). Scale bar = 50  $\mu$ m

**Table 2.** Extent of arbuscular mycorrhizal (AM), dark septate endophyte (DSE) fungal colonization in selected tree species in Aliyar, South India.

Family/ Plant species	Spore number 100 g <sup>-1</sup> soil <sup>a</sup>	AM colonization (%) <sup>b</sup>					DSE Colonization (%) <sup>c</sup>		
		RLH	RLA/RLAC	RLV	RLHC	RLTC	RLDSFH	RLM	RLDTC
Anacardiaceae									
<i>Mangifera indica</i>	10.00 ± 1.73 <sup>d-g*</sup>	5.67 ± 1.97 <sup>hi</sup>	23.23 ± 10.62 <sup>ab</sup>	---	38.53 ± 10.01 <sup>dc</sup>	67.43 ± 1.82 <sup>a</sup>	---	---	---
Apocynaceae									
<i>Alstonia scholaris</i>	7.67 ± 4.06 <sup>g</sup>	4.58 ± 0.94 <sup>hij</sup>	40.16 ± 3.31 <sup>bc</sup>	---	21.54 ± 1.30 <sup>fg</sup>	66.28 ± 1.36 <sup>ab</sup>	---	---	---
<i>Tabernaemontana heyneana</i>	11.00 ± 1.15 <sup>b-g</sup>	---	---	---	62.22 ± 7.90 <sup>a</sup>	62.22 ± 6.70 <sup>ab</sup>	---	---	---
<i>Wrightia tinctoria</i>	11.33 ± 3.84 <sup>a-g</sup>	---	5.30 ± 3.15 <sup>jk</sup>	4.49 ± 3.62 <sup>ef</sup>	51.02 ± 9.02 <sup>bcd</sup>	60.82 ± 2.84 <sup>ab</sup>	1.16 ± 0.58 <sup>b</sup>	5.15 ± 2.89 <sup>c</sup>	6.31 ± 3.47 <sup>bc</sup>
Bignoniaceae									
<i>Spathodea campanulata</i>	10.00 ± 2.65 <sup>d-g</sup>	4.62 ± 1.46 <sup>hij</sup>	49.44 ± 1.45 <sup>a</sup>	---	16.36 ± 1.75 <sup>g</sup>	70.42 ± 4.66 <sup>a</sup>	---	---	---
Bischofiaceae									
<i>Bischofia javanica</i>	9.67 ± 1.20 <sup>efg</sup>	9.23 ± 1.26 <sup>gh</sup>	47.55 ± 9.11 <sup>ab</sup>	6.53 ± 6.53 <sup>b-f</sup>	---	63.32 ± 4.40 <sup>ab</sup>	0.65 ± 0.05 <sup>b</sup>	7.19 ± 0.19 <sup>c</sup>	7.84 ± 0.84 <sup>bc</sup>
Bombacaceae									
<i>Bombax ceiba</i>	15.67 ± 0.88 <sup>a</sup>	18.95 ± 3.98 <sup>ab</sup>	3.98 ± 0.97 <sup>jk</sup>	9.23 ± 1.22 <sup>b-f</sup>	---	32.16 ± 5.02 <sup>c</sup>	---	58.65 ± 5.80 <sup>ab</sup>	58.65 ± 5.80 <sup>bc</sup>
Caesalpiniaceae									
<i>Peltophorum pterocarpum</i>	11.33 ± 1.45 <sup>a-g</sup>	---	---	5.43 ± 3.43 <sup>c-f</sup>	63.41 ± 3.65 <sup>a</sup>	68.85 ± 1.86 <sup>a</sup>	---	---	---
Combretaceae									
<i>Terminalia bellirica</i>	10.67 ± 1.86 <sup>c-g</sup>	---	2.78 ± 0.70 <sup>jk</sup>	---	65.04 ± 0.79 <sup>a</sup>	67.82 ± 1.49 <sup>a</sup>	---	---	---
Euphorbiaceae									
<i>Drypetes elata</i>	9.67 ± 1.76 <sup>efg</sup>	12.25 ± 1.57 <sup>d-g</sup>	51.60 ± 2.71 <sup>a</sup>	---	---	63.86 ± 2.77 <sup>ab</sup>	---	---	---
<i>Macaranga peltata</i>	7.67 ± 2.73 <sup>g</sup>	9.19 ± 2.50 <sup>gh</sup>	9.79 ± 2.10 <sup>ij</sup>	27.88 ± 9.31 <sup>a</sup>	18.12 ± 9.43 <sup>g</sup>	65.01 ± 2.28 <sup>ab</sup>	1.17 ± 0.17 <sup>b</sup>	5.46 ± 1.90 <sup>c</sup>	6.63 ± 2.60 <sup>bc</sup>
Lauraceae									
<i>Litsea monopetala</i>	15.00 ± 1.15 <sup>abc</sup>	6.19 ± 1.36 <sup>hi</sup>	25.49 ± 2.89 <sup>efg</sup>	---	31.26 ± 0.15 <sup>ef</sup>	62.96 ± 3.70 <sup>ab</sup>	---	3.39 ± 1.92 <sup>c</sup>	3.39 ± 1.92 <sup>c</sup>
<i>Litsea</i> sp.	13.00 ± 0.58 <sup>a-f</sup>	4.99 ± 0.50 <sup>hi</sup>	33.07 ± 0.98 <sup>c-f</sup>	---	30.38 ± 2.23 <sup>ef</sup>	68.44 ± 1.30 <sup>a</sup>	---	---	---
<i>Litsea stocksii</i>	12.33 ± 0.88 <sup>a-f</sup>	12.61 ± 3.37 <sup>def</sup>	28.82 ± 0.92 <sup>d-g</sup>	10.38 ± 4.84 <sup>b-c</sup>	9.97 ± 1.14 <sup>gh</sup>	61.78 ± 5.96 <sup>ab</sup>	---	5.73 ± 0.73 <sup>c</sup>	5.73 ± 0.73 <sup>b-c</sup>
Malvaceae									
<i>Thespesia populnea</i>	13.67 ± 1.76 <sup>a-c</sup>	16.75 ± 0.77 <sup>bcd</sup>	49.09 ± 3.72 <sup>a</sup>	---	---	65.84 ± 4.07 <sup>ab</sup>	---	---	---
Meliaceae									
<i>Aglaia</i> sp.	11.00 ± 2.03 <sup>b-g</sup>	14.66 ± 1.18 <sup>b-c</sup>	17.73 ± 8.64 <sup>fg</sup>	17.92 ± 6.03 <sup>b</sup>	---	51.80 ± 10.94 <sup>b</sup>	12.18 ± 2.18 <sup>ab</sup>	8.33 ± 0.33 <sup>c</sup>	23.08 ± 13.08 <sup>b</sup>
Mimosaceae									
<i>Acacia auriculiformis</i>	10.33 ± 2.40 <sup>d-g</sup>	---	---	6.13 ± 3.32 <sup>c-f</sup>	56.17 ± 5.11 <sup>abc</sup>	62.41 ± 1.30 <sup>ab</sup>	---	---	---
<i>Acacia dealbata</i>	11.67 ± 2.40 <sup>a-g</sup>	7.54 ± 1.47 <sup>ghi</sup>	4.28 ± 1.16 <sup>jk</sup>	12.93 ± 4.02 <sup>bcd</sup>	13.54 ± 3.84 <sup>g</sup>	38.29 ± 4.26 <sup>c</sup>	---	60.96 ± 2.43 <sup>a</sup>	60.96 ± 2.43 <sup>a</sup>
Moraceae									
<i>Ficus amplissima</i>	10.00 ± 2.08 <sup>d-g</sup>	14.41 ± 1.75 <sup>b-c</sup>	45.42 ± 5.71 <sup>ab</sup>	---	---	61.32 ± 3.57 <sup>ab</sup>	---	---	---
<i>Ficus microcarpa</i>	11.00 ± 1.15 <sup>b-g</sup>	---	---	---	58.64 ± 3.13 <sup>ab</sup>	58.64 ± 3.13 <sup>ab</sup>	---	---	---
<i>Ficus racemosa</i>	8.67 ± 2.03 <sup>fg</sup>	---	---	13.84 ± 1.38 <sup>bc</sup>	47.47 ± 3.68 <sup>cd</sup>	61.32 ± 4.87 <sup>ab</sup>	7.86 ± 5.03 <sup>ab</sup>	5.47 ± 2.84 <sup>c</sup>	13.33 ± 7.08 <sup>bc</sup>
<i>Ficus</i> sp.	14.00 ± 2.52 <sup>a-c</sup>	---	---	1.23 ± 1.23 <sup>ef</sup>	62.42 ± 5.07 <sup>a</sup>	63.66 ± 3.96 <sup>ab</sup>	---	---	---
<i>Strobilus asper</i>	10.33 ± 1.86 <sup>d-g</sup>	---	---	1.89 ± 1.89 <sup>ef</sup>	64.02 ± 1.66 <sup>a</sup>	66.09 ± 1.53 <sup>ab</sup>	0.64 ± 0.04 <sup>b</sup>	---	0.64 ± 0.04 <sup>c</sup>
Myrtaceae									
<i>Eucalyptus tereticornis</i>	12.33 ± 0.88 <sup>a-f</sup>	3.02 ± 0.82 <sup>ij</sup>	1.49 ± 0.75 <sup>jk</sup>	9.81 ± 3.54 <sup>b-f</sup>	---	14.32 ± 5.87 <sup>d</sup>	13.63 ± 7.19 <sup>ab</sup>	51.72 ± 5.50 <sup>b</sup>	65.34 ± 2.30 <sup>a</sup>
<i>Syzygium caryophyllatum</i>	12.33 ± 1.76 <sup>a-f</sup>	6.06 ± 2.53 <sup>hi</sup>	33.85 ± 5.85 <sup>cde</sup>	12.90 ± 5.52 <sup>bcd</sup>	15.85 ± 0.64 <sup>g</sup>	68.66 ± 1.86 <sup>a</sup>	1.11 ± 0.11 <sup>b</sup>	1.93 ± 0.13 <sup>c</sup>	3.04 ± 0.98 <sup>c</sup>
Oleaceae									
<i>Chionanthus mala-elengi</i>	11.33 ± 2.19 <sup>a-g</sup>	21.50 ± 1.66 <sup>a</sup>	35.09 ± 3.64 <sup>cd</sup>	4.17 ± 2.08 <sup>ef</sup>	---	60.76 ± 6.95 <sup>ab</sup>	13.02 ± 3.02 <sup>ab</sup>	1.23 ± 0.63 <sup>c</sup>	14.25 ± 3.20 <sup>bc</sup>
Papilionaceae									
<i>Dalbergia latifolia</i>	15.33 ± 1.45 <sup>ab</sup>	11.46 ± 2.83 <sup>efg</sup>	45.77 ± 3.48 <sup>a</sup>	7.24 ± 2.80 <sup>b-f</sup>	---	64.46 ± 0.93 <sup>ab</sup>	---	---	---
<i>Pongamia pinnata</i>	10.33 ± 2.33 <sup>d-g</sup>	5.17 ± 2.22 <sup>hi</sup>	5.25 ± 1.09 <sup>jk</sup>	2.17 ± 1.35 <sup>ef</sup>	40.31 ± 9.00 <sup>dc</sup>	52.90 ± 12.85 <sup>b</sup>	17.74 ± 1.74 <sup>a</sup>	0.54 ± 0.04 <sup>c</sup>	18.28 ± 1.28 <sup>bc</sup>
<i>Pterocarpus santalinus</i>	12.67 ± 1.20 <sup>a-f</sup>	---	---	7.27 ± 3.75 <sup>b-f</sup>	54.08 ± 5.63 <sup>abc</sup>	61.35 ± 3.32 <sup>ab</sup>	---	---	---
Rutaceae									
<i>Clausena dentata</i>	12.33 ± 0.88 <sup>a-f</sup>	13.83 ± 1.66 <sup>c-f</sup>	46.42 ± 8.63 <sup>a</sup>	---	---	60.26 ± 5.88 <sup>ab</sup>	---	---	---
<i>Glycosmis cymosa</i>	10.67 ± 1.45 <sup>c-g</sup>	12.44 ± 1.45 <sup>d-g</sup>	34.12 ± 0.80 <sup>cde</sup>	---	20.30 ± 2.78 <sup>fg</sup>	66.86 ± 1.91 <sup>ab</sup>	---	---	---
<i>Glycosmis pentaphylla</i>	14.33 ± 1.86 <sup>a-d</sup>	14.85 ± 2.87 <sup>b-c</sup>	51.23 ± 2.47 <sup>a</sup>	---	---	66.08 ± 0.92 <sup>ab</sup>	---	---	---
<i>Murraya paniculata</i>	14.33 ± 1.20 <sup>a-d</sup>	---	---	3.85 ± 3.02 <sup>def</sup>	64.24 ± 1.54 <sup>a</sup>	68.09 ± 1.05 <sup>a</sup>	---	---	---
Salicaceae									
<i>Salix tetrasperma</i>	12.00 ± 1.73 <sup>a-g</sup>	17.91 ± 2.59 <sup>abc</sup>	45.35 ± 1.44 <sup>ab</sup>	---	---	63.27 ± 3.40 <sup>ab</sup>	---	---	---
Verbenaceae									
<i>Clerodendrum viscosum</i>	13.33 ± 1.86 <sup>a-c</sup>	6.27 ± 0.72 <sup>hi</sup>	15.63 ± 1.15 <sup>hi</sup>	7.60 ± 4.88 <sup>b-f</sup>	39.88 ± 4.56 <sup>dc</sup>	69.37 ± 0.14 <sup>a</sup>	---	---	---

<sup>a</sup>Spore numbers 100 g per soil; <sup>b</sup>RLH, root length with hyphae, RLA/RLAC, root length with arbuscules/arbusculate coils, RLV, root length with vesicles, RLHC, root length with hyphal coils, RLTC, root length with total colonization; <sup>c</sup>RLDSFH, root length with dark septate fungal hyphae, RLM, root length with moniliform hyphae, RLDTC, root length with DSE total colonization. \*Mean ± S.E. Means in a column followed by the same superscript(s) do not significantly differ ( $p > 0.05$ ) according to DMRT.

The extent of DSE colonization ranged between < 1% (*Streblus asper*, Moraceae) and 65% (*Eucalyptus tereticornis*, Myrtaceae) (Table 2). Majority of the plants (50%) with DSE fungal association had colonization levels around 10%. The percentage root length with DSE fungal hyphae (%RLDSFH) ranged between < 1% (*Streblus asper*, Moraceae) and 17 % (*Pongamia pinnata*, Papilionaceae). Similarly, percentage root length with moniliform hyphae (%RLM) ranged between < 1% (*P. pinnata*, Papilionaceae) and 61% (*Acacia dealbata*, Mimosaceae) (Fig. 1 i, j). The extent of total DSE colonization was inversely related to total AM colonization (Fig. 2).



**Fig 2.** Relationship between arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal colonization in trees of Aliyar

#### AM fungal spores

A total of 11 AM fungal morphotypes were distinguished based on spore morphology, and morphotypes could be identified to the species level. These included *Acaulospora scrobiculata* Trappe, *Acaulospora spinosa* C. Walker & Trappe, *Glomus albidum* C. Walker & L.H. Rhodes, *Glomus aggregatum* N.C. Schenck & G.S. Sm., *Glomus microaggregatum* Koske, Gemma & P.D. Olexia, *Glomus deserticola* Trappe, Bloss & J.A. Menge, *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe, *Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker, *Glomus ambisporum* G. S. Sm. & N.C. Schenck, *Glomus sinuosum* (Gerd. & B.K. Bakshi) R.T. Almeida & N.C. Schenck and *Glomus viscosum* T.H. Nicolson. AM fungal spores in the soils ranged from 7 spores per 100 g soil (*Alstonia scholaris*, Apocynaceae) to 16 spores per 100 g soil (*Bombax ceiba*, Bombacaceae). The extent of AM colonization was not related to AM fungal spore numbers ( $r = 0.094$ ,  $p > 0.05$ ;  $n = 35$ ).

## Discussion

AM fungi play a vital role in natural eco-systems like tropical forestry management (Mukerji et al. 1996) by influencing the composition and succession of plants (Janos 1980; Giovannetti and Gianinazzi-pearson 1994) through increasing the volume of soil explored by tree roots (Dhar and Mridha 2006). The major attributes of AM fungal associations include P uptake (Mar-

schner and Dell 1994), nutrient cycling (Finlay 2004), and exudates in the mycorrhizosphere (Linderman 1988). The soil examined in this study were low in available nutrients as reported for the most tropical soils (Muthukumar and Udaiyan 2000; Muthukumar et al. 2006). According to Trappe (1987), Harley and Harley (1987) and Wang and Qiu (2006) only around 3% of the known land plant species have been examined for their mycorrhizal status. This is the first evaluation of the mycorrhizal status of 20 tree species examined. The presence of AM association in all the tree species indicates the widespread occurrence of mycorrhizae in tree species.

The extent of AM colonization observed in the present study (14%–70%) is lower compared to the earlier (17%–100%) report by Khade and Rodrigues (2003) for tree species from Western Ghats region of Goa. However, the average of AM fungal colonization in tree species (61%) is higher than the observation (50.7%) of Khade and Rodrigues (2003). In the present study, 64% of root length was colonized by AM fungi in *Ficus* sp. contrasting the observation of Tawaraya et al. (2003) where only 15% of the root length was colonized by AM fungi in *Ficus* species growing in peat swamp forest of Indonesia. The tree species *Macaranga peltata* had 65% of its root length colonized by AM fungi, but Khade and Rodrigues (2003) reported a higher colonization (89.98%) for this species. *Ficus racemosa* had 61% of colonization in this study, but Nandakwang et al. (2008) reported a lower colonization of the same species in (33%) natural evergreen forest compared to 98% in restoration forest in dry tropical forest in northern Thailand. However, the extent of colonization observed in *A. auriculiformis* in the present study is almost similar to the observation of Dhar and Mridha (2006). The tree *A. auriculiformis* has been reported to be mycorrhizal (Lakshman et al. 2001; Dhar and Mridha 2006; Nandi et al. 2009) and non mycorrhizal (Muthukumar and Udaiyan 2000) because members of mimosaceae adapted for ectomycorrhizal association are rarely colonized by AM fungi. The root length colonization of AM in *Mangifera indica* in the present study (67%) is almost similar to the findings of Muthukumar et al. (2006), but contrast the observations of Lakshman et al. (2001) and Khanam (2007) who observed 25% and 30% root colonization for this species respectively from deciduous forest of Karnataka, India and horticultural farm of Bangladesh. Variations in edapho-climatic factors and different microenvironments might be responsible for the variation in AM fungal colonization development in the host trees and the presence of spore populations in the different sites (Mridha and Dhar 2007).

In this study, AM morphology was categorised for all the tree species compared to previous studies evaluating only the mycorrhizal status of tree species (Khade and Rodrigues 2003; Dhar and Mridha 2006). AM morphological type is determined by the presence of continuous longitudinal air-spaces in the root cortex (Brundrett and Kendrick 1990). In addition, Smith and Smith (1997) and Cavagnaro et al. (2001) reported the effect of fungal identity on determination of AM fungal morphology in wild tomato plants. AM morphology appeared to depend more on the characteristics of plants rather than those of fungi (Yamato and



Iwasaki 2002; Matekwor Ahulu et al. 2007). Further factors that affect plant growth, including soil factors, may also contribute to morphological variability observed (Dickson 2004; Dickson et al. 2007). In this study, six of the 35 tree species had *Arum*-type AM morphology. In contrast, Wubet et al. (2003) found that AM in roots of all the 11 indigenous trees they examined from dry afro-montane forests of Ethiopia were of *Arum*-type. Fisher and Jayachandran (2005) suggested that each plant species has a particular pattern of AM colonization, which is regulated by the host. Fracchia et al. (2009) reported that most of the tree species they examined for mycorrhizal status from Chaco-Serrano woodland had both *Arum*- and *Paris*-type AM morphology. However, the predominance (18 tree species) of intermediate-type AM morphology in the present study emphasizes the need for screening more tree species from various geographical conditions.

The lack of correlation between AM colonization and spore numbers agree with the observation of Rani et al. (1995). As AM fungal colonization and sporulation are influenced by a wide array of plant, fungal and environmental factors they need not be related. Further, infective propagules of AM fungi include not only the spores, but also extramatrical hyphae in the soil and mycorrhizal roots of conspecifics (Smith and Read 2008). The rhizosphere soils of tree species harboured spore morphotypes of only 11 AM fungal species, which is almost similar with the observations of Dhar and Mridha (2006) who reported only 6 AM fungal spore morphotypes associated with trees of Madhupur forest, Bangladesh.

The average of AM fungal spore numbers (7–16 spores 100 g<sup>-1</sup> soil) recorded in this study is low compared to those recorded (18–745 spores 100 g<sup>-1</sup> rhizosphere soil) in the rhizosphere soils of different tree species from Western Ghats of Goa, India (Khade and Rodrigues 2003). Nandakwang et al. (2008) reported that spore density of natural forest was the minimum compared to restoration forest because of the high root density which negatively influenced AM fungal sporulation, as in our study. Raman et al. (1992) reported 30–301 spores 100 g<sup>-1</sup> rhizosphere soil in Mamandur forest of Tamil Nadu. In Malaysian region, Chulan and Ragu (1986) had reported very high counts of 45,860 spores 100 g<sup>-1</sup> soil under the oil palm. Sporulation is triggered during the period of fungal resource mobilization from senescing roots (Sutton and Barron 1972) and when root activity is interrupted by a long dry season (Janos 1980). In forest ecosystems generally trees produce new roots throughout the year, and spores may germinate and colonize these newly formed roots. This may be one of the reasons for low spore counts observed in this study. Another reason for low spore numbers in this study may be the time of sampling, which was during the rainy season. Nandi et al. (2009) also reported large spore numbers before the rainy season than during the rainy season in forest trees of Bangladesh. The predominance of species belonging to *Glomus* is in accordance with observations where species of *Glomus* tend to dominate tropical soils (Ragupathy and Mahadevan 1993; Muthukumar et al. 2003; 2006; Khade and Rodrigues 2003; Sathiyadash et al. 2010). In this study, 82% of the AM species isolated were in *Glomus* compared to the 63% (15 of 24) reported by Nandakwang et al. (2008) for in tropical forest in Thailand.

DSE associations were observed for the first time in roots of 14 tree species belonging to 11 plant families. In the present study, 40% of the trees had dual colonization of DSE and AM. Such dual association has already been reported in various forest types (Chaudhry et al. 2009; Muthukumar et al. 2003; 2006). This leads to the hypothesis that DSE may act as a backup system of AM fungi if the condition unfavorable for AM fungal development or any stressful conditions (Barrow 2003). Micro-sclerotia were observed in the root cortical cells and their frequency of occurrence varied with the plant species. No fungal structures were found in the root stelar region of any of the plants examined, which contrasts with reports by Yu et al. (2001) where occasional penetration of the vascular tissue by fungi has been observed. This is expected since dark septate endophytes are wide spread among angiosperms (Jumpponen and Trappe 1998). In spite of the assumption of the widespread occurrence of DSE in angiosperms, their occurrence has been reported in only 59 tropical plant species (Jumpponen and Trappe 1998).

The negative relationship between DSE and AM fungal colonization is in agreement with the observations of Chaudhary et al. (2006) and Muthukumar and VEDIYAPPAN (2010) where a similar relationship has been observed between these variables. Although no experimental evidence exists for a direct interaction between these fungal groups occupying the same niche, circumstantial evidence does suggest the role of environmental factors in altering the presence or proportion of these fungal groups within host roots. DSE fungi have been observed to dominate root colonization under conditions that are not conducive for AM formation (Read et al. 1976; Haselwandtler and Read 1982). Christie and Kilpatrick (1992) speculated that the transition from AM to DSE fungal colonization could be perceived as an indicator of increasing environmental stress. However, the specific factors influencing this transition depends on plant community concerned (Christie and Kilpatrick 1992). The results of the present study clearly demonstrate that tree species are hosts for AM fungi and some also hosts DSE fungi. Although the role of AM fungi on tree growth and health is adequately demonstrated, further work is required to understand the potential role of DSE fungi in the growth and development of trees. Further, experimental studies are needed in future to ascertain the nature of interaction between DSE and AM fungi in co-occurring situations.

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